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Investigation of the antimicrobial effect of endemic *Sideritis galatica* plant

Ulaş ACARÖZ^{1,a*}, Sinan İNCE^{2,b}, Recep KARA^{1,c}, Zeki GÜRLER^{1,d}, Ali SOYLU^{1,e}

¹ Afyon Kocatepe University, Veterinary Faculty, Department of Food Hygiene and Technology, Afyonkarahisar, Turkey

² Afyon Kocatepe University, Veterinary Faculty, Department of Pharmacology and Toxicology, Afyonkarahisar, Turkey

ORCID: 0000-0002-1533-4519^a; 0000-0002-1915-9797^b; 0000-0002-9257-7506^c; 0000-0002-9037-2945^d; 0000-0003-3199-782X^e

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ABSTRACT:

Herbal teas are widely consumed in Turkey. This study aimed to evaluate the antimicrobial activity of methanol, acetone, and aqueous extracts of endemic *Sideritis galatica* plant collected from Afyonkarahisar. For this purpose, the effect of different concentrations (2.5%, 5%, 10%, 20%) of these extracts on *L. monocytogenes*, *B. cereus*, *S. aureus*, *E. faecalis*, *E. coli*, *E. coli O157*, *S. typhimurium*, *C. albicans* were investigated by the disk diffusion method. Methanol extract showed antimicrobial activity against all of the tested microorganisms. Also, acetone and aqueous extracts showed antimicrobial activity against all of the tested microorganisms except *E. faecalis* (for acetone extract), *E. coli* and *E. coli O157* (for aqueous extract). Consequently, *S. galatica* as an endemic plant showed antimicrobial activity especially at high concentrations against tested microorganisms.

Endemik Sideritis galatica bitkisinin antimikrobiyal etkisinin araştırılması

ÖZET:

Bitkisel çaylar Türkiye'de yaygın olarak tüketilmektedir. Bu çalışmada Afyonkarahisar'dan toplanan endemik *Sideritis galatica* bitkisinin metanol, aseton ve sulu ekstraktlarının antimikrobiyal aktivitesi değerlendirildi. Bu amaçla, bu ekstraktların farklı konsantrasyonlarının (% 2,5, % 5, % 10, % 20) *L. monocytogenes*, *B. cereus*, *S. aureus*, *E. faecalis*, *E. coli*, *E. coli O157*, *S. typhimurium*, *C. albicans* disk difüzyon yöntemi ile araştırıldı. Metanol ekstraktı test edilen tüm mikroorganizmalara karşı antimikrobiyal aktivite gösterdi. Ayrıca, aseton ve sulu ekstraktlar, *E. faecalis* (aseton ekstraktı için), *E. coli* ve *E. coli O157* (sulu ekstrakt için) hariç test edilen tüm mikroorganizmalara karşı antimikrobiyal aktivite gösterdi. Sonuç olarak, endemik bir bitki olarak *Sideritis galatica*, test edilen mikroorganizmalara karşı özellikle yüksek konsantrasyonlarda antimikrobiyal aktivite gösterdi.

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* Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address: ulasacaroz@aku.edu.tr

1. Introduction

Sideritis genus of *Labiatae* family possesses more than 150 different species and they are located at the Northern Hemisphere from the Western China to Bahamas. Many of them are largely distributed in the Mediterranean area. In particular, Turkey and Spain have a lot of different species. While predominate areas of Spain are Canary Islands and the Southeast of Iberian Peninsula, most species in Turkey found to be in Marmara and Aegean regions (1, 6, 10). 25 *Sideritis* species are endemic to Turkey and some of them have been exported (15). This genus mainly grows in mountainous areas (over 800-1.000 m) (8). The origin of *Sideritis* genus comes from the Greek word "sideros". Its meaning is iron because these plants are used to treat wounds caused by metal weapons in the ancient times (4).

Sideritis species are traditionally employed as flavoring agents, teas, and for medicinal purposes in the Mediterranean and Balkan regions and also in Turkey (13). They recently became quite popular and found in several of shops. They are called and marketed as malotira, té de Puerto, mountain tea, ada çayı, dağ çayı. This herb is found to be whole or in the cut form (8, 15).

Traditional medicine uses the species of this genus for centuries due to their anti-inflammatory, anticonvulsant, antiulcerative, antispasmodic, analgesic, antioxidant, and antimicrobial properties (5, 8). These effects are related to various compounds of this genus such as terpenes, flavonoids, essential oil, lignanes, iridoids, coumarins and sterols. In addition, almost every species contain essential oils, flavonoids, and diterpenes (4).

The present study investigated antibacterial and antifungal activities of Turkish endemic *Sideritis galatica* plant by evaluating its water, methanol and acetone extracts against eight bacteria and yeasts including *L. monocytogenes*, *B. cereus*, *S. aureus*, *E. faecalis*, *E. coli*, *E. coli O157*, *S. typhimurium*, *C. albicans*.

2. Material and Methods

Plant Material:

Sideritis galatica samples were collected from Şuhut districts of Afyonkarahisar of Turkey in June 2017. This plant is registered at the Herbarium of Biology Department of Afyon Kocatepe University with Herbarium number of AKU-8315.

Extraction:

Firstly, a drying process of the plant was performed at shadow for its aerial parts. Then, water, methanol and acetone extracts of *Sideritis galatica* were prepared as previously described by Knörle et al. (8) with some modifications. Briefly, dried herb samples were ground to powder for 2 min by a grinder. Powdered samples were dissolved in different solvents including water, acetone and methanol as extracting agent. A filtration step was performed to the obtained extracts and they were dried by rotary evaporation. The resulted extracts were expressed as water, acetone, methanol extracts.

Microorganism strains and determination of antimicrobial activity:

The microorganism strains employed in the study were presented in Table 1. Antimicrobial activity of the tested microorganisms was determined by disc diffusion method. For that aim, a suspension containing tested microorganisms with a concentration of 10^6 - 10^7 cfu/ml was prepared and then the related microorganism spread on the solid media plates. Then 20 µL of the *Sideritis galatica* extracts were impregnated to the 6-mm diameter paper discs and they were placed on the inoculated agar. An incubation period followed this step for bacterial strains (37°C, 18-24 h) and yeast (27°C, 24-48 h).

Table 1: The microorganism strains employed in the study**Tablo 1:** Çalışmada kullanılan mikroorganizma suşları

Microorganism Strains	ATCC Strain Number
<i>B. cereus</i>	ATCC 11778
<i>C. albicans</i>	ATCC 90028
<i>E. faecalis</i>	ATCC 29212
<i>E. coli</i>	ATCC 25292
<i>E. Coli O157</i>	ATCC 43894
<i>L. monocytogenes</i>	ATCC 7644
<i>S. aureus</i>	ATCC 25923
<i>S. typhimurium</i>	ATCC 14028

3. Results

The activities of water, methanol and acetone extracts of *Sideritis galatica* at the concentrations of 2.5 %, 5 %, 10 %, 20 % against the tested gram-positive bacteria strains and *C. albicans* were given in Table 2 and gram-negative bacteria were given in Table 3. The study results showed that water extract of *Sideritis galatica* showed activity against *C. albicans* at all tested concentrations. In addition, water extract did not exhibit any activity against other tested seven bacteria strain at the 2.5 %, and 5 % concentrations whereas higher concentration of water extract (20%) showed antibacterial activity against all tested microorganism except *E. coli* and *E. coli O157*. When the methanol extract evaluated, the highest concentration (20%) of this extract possessed antibacterial and antifungal activity against all tested microorganisms. However, the lower concentrations of methanol extract were not effective except *L. monocytogenes* (5% and 10%) and *S. aureus* (10%). Similarly, highest concentration of acetone extract was effective against all tested microorganisms except *E. fecalis*. Only 10 % of acetone extract showed activity against *B. cereus* and *C. albicans* while the other concentrations were ineffective.

Table 2: Antimicrobial activities of water, methanol and acetone extracts of *Sideritis galatica* (2.5, 5, 10, and 20%) against Gram-positive bacteria and *C. albicans*.**Tablo 2:** *Sideritis galatica*'nın (% 2,5, 5, 10 ve 20) su, metanol ve aseton ekstraktlarının Gram pozitif bakterilere ve *C. albicans*'a karşı antimikrobiyal aktiviteleri.

	Water				Methanol				Acetone			
	2.5	5	10	20	2.5	5	10	20	2.5	5	10	20
<i>L. monocytogenes</i>	-	-	-	15	-	10	13	14	-	-	-	14
<i>B. cereus</i>	-	-	-	14	-	-	-	15	-	-	10	14
<i>S. aureus</i>	-	-	10	11	-	-	12	15	-	-	-	12
<i>C. albicans</i>	10	11	12	13	-	-	-	14	-	-	10	13

The values express zone of inhibition (mm), -: no inhibition

Table 3: Antimicrobial activities of water, methanol and acetone extracts of *Sideritis galatica* (2.5, 5, 10, and 20 %) against Gram-negative bacteria.

Tablo 3: *Sideritis galatica*'nın (% 2,5, 5, 10 ve 20) su, metanol ve aseton ekstraktlarının Gram pozitif bakterilere ve *C. albicans*'a karşı antimikrobiyal aktiviteleri.

	Water				Methanol				Acetone			
	2.5	5	10	20	2.5	5	10	20	2.5	5	10	20
<i>E. faecalis</i>	-	-	-	11	-	-	-	12	-	-	-	-
<i>E. coli</i>	-	-	-	-	-	-	-	16	-	-	-	13
<i>E. coli O157</i>	-	-	-	-	-	-	-	15	-	-	-	12
<i>S. typhimurium</i>	-	-	-	10	-	-	-	11	-	-	-	10

The values express zone of inhibition (mm), -: no inhibition

4. Discussion and Conclusion

Many researchers have investigated the antimicrobial activities of different *Sideritis* species. Sagdic et al. (12) determined the antimicrobial activities of the methanol extracts of *S. ozturkii* and *S. caesarea* which are endemic plants for Turkey. They employed agar diffusion method against fifteen microorganisms for four different concentrations (1, 2.5, 5, 10 %) of these extracts. They reported that the extracts of both plants were least effective at lower concentrations (1 % and 2.5 %) and generally yeast strains were found to be more resistant than tested bacterial strains. Their antimicrobial activities changed related to the tested microorganisms and the highest concentration (10 %) was reported as the most active of the concentrations against all of the tested microorganisms. In another study, *Sideritis* essential oils were reported to show both antifungal and antibacterial activities (11). The extent of the antibacterial activity of the extracts of *Sideritis* genus could be attributed to their phenolic content such as diterpenoids and flavonoids (2). Also, Iscan et al. (7) obtained essential oils from *Sideritis cilicica* and *Sideritis bilgerana* and performed their analysis by GC and GC/MS. Their results showed that major constituents in the *S. cilicica* oil were found to be as β -phellandrene (20%), α -pinene (28%), and β -Pinene (39%) whereas the main components in the *S. bilgerana* oil were determined as α -pinene (32%), and β -pinene (48%). To evaluate antimicrobial activities of these oils, microdilution broth method was used and both oils were reported to show good inhibitory effects on *C. albicans*. Dulger et al. (3) investigated the activity of the methanol extracts of seven endemic *Sideritis* species for Turkey were (30 mg/ml) on clotrimazole-resistant *C. albicans*. *S. trojana* and *S. bilgerana* were reported to be most active plants against *C. albicans*. Temel et al. (15) reported that different levels of water extracts of *Sideritis akmanii* showed significant activity on several bacteria including *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*. However, it did not exhibit any antimicrobial activity against yeasts. Koutsaviti et al (9) evaluated the antibacterial activity of five taxa of Greek *Sideritis* by broth microdilution method against five gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Bacillus subtilis*) and three gram bacteria strains (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*), and two different strains of *Candida albicans*. They used hydrodistillation to obtain extracts of the related *Sideritis* species. Considerable antibacterial and antifungal activity against certain microorganism strains were reported. Even, the MIC values of *S. lanata* against *M. luteus* and *S. aureus* were comparable to reference antibiotics. Tadić et al. (13) reported about the antimicrobial activity of *Sideritis romana* L. subsp. *purpurea* in a recent study. In their study, *Candida albicans*, methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant and carbapenem-susceptible *Klebsiella pneumoniae*, and

Escherichia coli were employed as test microorganisms. The obtained essential oil was highly potent against both methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* strains. In addition, 1,2-dichloroethane and n-hexane extracts of the plant was reported to show a potent fungicidal activity. In another study, antimicrobial effects of *Sideritis scardica* extracts were determined by Tadić et al. (14). According to the results of their study, ethanolic extract of the plant showed various level of antimicrobial activity all tested strains which were *Micrococcus luteus*, *Candida albicans*, *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Also, they have stated that maximum activity was seen against *M. luteus*, *S. epidermidis*, *P. aeruginosa* and *E. coli* while moderate activity was observed against *K. pneumoniae*.

Different *Sideritis* species were reported to show various level of antimicrobial activity against tested bacteria and yeast strains. This may be caused by the different active ingredients of the respective *sideritis* species which could be affected by several factors such as harvesting time of the herb and extraction procedure.

In conclusion, three different extracts of *Sideritis galatica* including water, methanol, acetone extracts were evaluated for the antimicrobial activity. Although lower extract concentrations were not so effective against tested microorganisms, higher concentrations especially 20 % concentrations of the extracts were able to show antimicrobial activity. However, water extract did not show activity against *E. coli* and *E. coli O157* while acetone extract was not able to show any activity against *E. faecalis*.

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Conflict of Interest

The author declared no conflict of interest.

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Authors' Contributions

Idea / concept: Ulaş ACARÖZ, Sinan İNCE

Experiment design: Ulaş ACARÖZ, Sinan İNCE, Recep KARA

Supervision / Consultancy: Ulaş ACARÖZ, Sinan İNCE

Data collecting: Ulaş ACARÖZ, Sinan İNCE, Recep KARA, Zeki GÜRLER, Ali SOYLU

Data analysis and interpretation: Ulaş ACARÖZ, Sinan İNCE, Recep KARA, Zeki GÜRLER

Literature search: Ulaş ACARÖZ, Sinan İNCE, Zeki GÜRLER, Ali SOYLU

Writing the article: Ulaş ACARÖZ, Sinan İNCE

Critical review: Ulaş ACARÖZ, Sinan İNCE, Recep KARA

Ethical Approval

An ethical statement was received from the authors that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules.

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